Ultrastructural and Light Microscopic Observations of Mature Epididymal Spermatozoa and Sperm Maturation of the Greater Bilby, *Macrotis lagotis* (Metatheria, Mammalia)

*Stephen JOHNSTON *, Lina DADDOW ** & Frank CARRICK ***

* Department of Farm Animal Medicine and Production,
University of Queensland Veterinary Science Farm, Pinjarra Hills, Q 4069, Australia
** Zoology Department, The University of Queensland, Brisbane, Q 4072, Australia

ABSTRACT

Light microscopic and ultrastructural observations were made on mature *Macrotis lagotis* cauda epididymal spermatozoa. Sperm nuclear length measures 13.2 µm, midpiece length 16.2 µm and total sperm length 149.4 µm. The sperm head is simple and without lateral concavities typical of the Peramelidae. Parachromatin-like material is present as a thin layer on the dorsal nuclear surface and the lateral margins of the sperm head are uncondensed and pitted. The acrosome covers 2/5 of the dorso-rostral nuclear surface, extending caudally. The mitochondrial sheath is essentially round but slightly flattened in transverse section and sculptured on its inner surface to accommodate dense outer fibres. These fibres are widely separated in the caudal region of the midpiece and connecting lamellae doubled. The annulus is typically perameloid in structure. Unique cauda epididymal sperm characteristics include: lattice substructural material about the neck region and on the caudo-ventral inner surface of the nucleus; arcs of double thickened membranes underlying the plasma membrane near the caudal midpiece and the neck region of axoneme bifurcate about the proximal centriole. Evidence of sperm maturation during epididymal transit includes: dislocation and cranial migration of the neck insertion from a primary implantation fossa to a secondary insertion at the inner tip of the nucleus; slight acrosomal compaction; slight rotation of the sperm head parallel to the longitudinal axis of the sperm and the shedding of a cytoplasmic droplet. Although no cladistic analysis was attempted, the unique characteristics of *M. lagotis* spermatozoa support the present taxonomic status of the Thylacomyidae as a distinct family. In addition, shared spermatological characteristics between that of the Thylacomyidae, Dasyuridae, Peramelidae and *Tarsipes rostratus* are also evidence of a close phylogenetic relationship. However, the precise phylogeny of the Thylacomyidae requires further investigation.

RÉSUMÉ

Observations en microscopie photonique et électronique sur le spermatozoïde mûr de l'épididyme et sa maturation chez le grand Bilby *Macrotis lagotis* (Metatheria, Mammalia).

Des observations en microscopie photonique et électronique ont été faites sur les spermatozoïdes de la queue de l'épididyme de *Macrotis lagotis* (Bilby ou Grand Lièvre Marsupial). Les longueurs du noyau, de la pièce intermédiaire et du spermatozoïde sont de 13.2, 16.2 et 149.4 µm. La tête du spermatozoïde est simple et sans les cavités latérales typiques des Peramelidae. Un matériel de type parachromatinien est présent sous forme d'une couche fine sur la surface dorsale du noyau et les marges latérales de la tête ne sont pas condensées et sont alvéolées. L'acrosome couvre les deux cinquièmes de

JOHNSTON , S., DADDOW, L., & CARRICK, F., 1995. — Ultrastructural and light microscopic observations of mature epididymal spermatozoa and sperm maturation of the Greater Bilby, *Macrotis lagotis* (Metatheria, Mammalia). *In*: JAMIESON, B. G. M., AUSIO, J., & JUSTINE, J.-L. (eds), Advances in Spermatozoal Phylogeny and Taxonomy. *Mém. Mus. natn. Hist. nat.*, **166** : 397-407. Paris ISBN : 2-85653-225-X.

la surface nucléaire dorso-rostrale et s'étend vers la queue. La gaine mitochondriale est essentiellement ronde mais légèrement aplatie en coupe transversale et sculptée sur sa surface interne pour laisser la place aux fibres denses externes. Ces fibres sont largement séparées dans la région caudale de la pièce intermédiaire et les lamelles de connexion sont doublées. L'annulus a une structure pérameloïde typique. Les caractéristiques originales du spermatozoïde épididymaire sont: un matériel à structure de treillis autour de la région du cou et sur la surface intérieure caudo-ventrale du noyau; des arcs de membrane à double épaisseur soulignant la membrane plasmique près de la partie caudale de la pièce intermédiaire; une bifurcation de la région du cou de l'axonème près du centriole proximal. Les preuves de la maturation du spermatozoïde pendant le transit épididymaire sont: la dislocation et la migration vers la tête de l'insertion du cou à partir d'une fossette primaire jusqu'à une insertion secondaire à l'extrémité interne du noyau; une légère compactage de l'acrosome; une légère rotation de la tête du spermatozoïde parallèlement à l'axe longitudinal du spermatozoïde; la perte d'une goutte cytoplasmique. Bien qu'une analyse cladistique n'ait pas été tentée, les caractéristiques originales du spermatozoïde de *M. lagotis* soutiennent la position actuellement reconnue des Thylacomyidae comme une famille distincte. De plus, les caractéristiques spermatologiques partagées des Thylacomyidae, Dasyuridae, Peramelidae and *T. rostratus* sont la preuve de relations phylogéniques proches. Toutefois, la phylogénie précise des Thylacomyidae demande d'autres études.

While spermiogenesis of *Perameles nasuta* has been thoroughly documented, [19-23], observations of perameloid sperm maturation and mature spermatozoa are limited [14]. HARDING *et al.* [14] noted that most descriptions of epididymal sperm structure in the Peramelidae were incomplete or were included as scattered adjuncts in comparative studies [2, 3, 5, 6, 9-12, 16]. Most studies have examined spermatozoa from *P. nasuta* and *Isoodon macrourus*, with only preliminary observations reported for *P. gunni*, *I. obesulus* and *Echymipera rufescens* spermatozoa.

HARDING *et al.* [14] previously described the ultrastructure and epididymal maturation of *P. nasuta* and *I. macrourus* spermatozoa. These authors noted that mature spermatozoa from both species were similar but also displayed a number of distinct apomorphies. They concluded that ultrastructural changes during sperm maturation were not so obvious when compared to other marsupials, except for the dislocation and marked relocation of the neck from its original implantation fossa to a position close to the rostrum of the nucleus.

Preliminary observations made on *Perorcytes longicauda* and *Echymipera kalubae* sperm have indicated that these species conform to the distinctive perameloid pattern with only minor differences in size and shape [14]. The flagellar structure of *M. lagotis* sperm has also been described by HARDING *et al.* [14] who noted that it conformed to the basic perameloid pattern. However, poor fixation of testicular tissue from one animal, and the occurrence of degenerate spermatozoa from another old male, meant that detailed observations of head structure and to a lesser extent midpiece structure were not possible.

Sperm structure is regarded as a conservative taxonomic character, reflecting evolutionary affinities in the Marsupialia [24]. A detailed evaluation of the ultrastructure and epididymal maturation of *M. lagotis* should, therefore, provide valuable insights into the controversial phylogenetic position of the genus *Macrotis*, which to date has been based on skull and dental characters [8], chromosomal [4] and serological evidence [1].

The present paper describes for the first time the ultrastructure of the mature *Macrotis lagotis* spermatozoon as well as morphological changes during sperm maturation. Light microscopic observations of sperm dimensions are recorded.

MATERIALS AND METHODS

A six year old sexually mature captive male *Macrotis lagotis* located at Western Plains Zoo, Dubbo (32°15′S., 148°37′E.), Australia, was euthanased because of a prolonged chronic staphylococcal infection of the hind limbs. The animal was in poor condition and considered surplus breeding stock.

Under general gaseous anaesthesia (Halothane), the caput and cauda epididymides of one testis were removed and diced into 2mm x 2mm blocks and immersed into cold 3 % glutaraldehyde in 0.1 M phosphate buffer with 6% sucrose for approximately 1 h. Samples were then transferred into 0.1 M sucrose in phosphate buffer and transported back to the laboratory (The University of Queensland, St. Lucia) within 24 hours. Samples for electron microscopy were rinsed in phosphate buffer, followed by post-fixation for 80 min in 1% osmium tetroxide in 0.1 M phosphate buffer with 6%

sucrose. Specimens were then washed in buffer, dehydrated through an ascending ethanol series, infiltrated and embedded in SPURR's low viscosity epoxy resin. Ultrathin sections, 60 to 80 nm, were cut on an LKB 2128 UM IV ultramicrotome. Sections were collected on carbon stabilised colloidion coated 200 mesh and single slot grids and stained with REYNOLD's lead citrate [18], 6% uranyl acetate and further lead citrate [7]. All micrographs were taken on a Hitachi H-300 electron microscope at 75KV.

The epididymidis of the remaining testis was then dissected into warm saline and gently teased to release motile spermatozoa. Nigrosin - eosin stained spermatozoa were used to measure the midpiece and total sperm length via bright-field microscopy at 400 x. As the marsupial sperm nucleus decondenses on air drying [6], measurements of head length and width were made on formalin fixed spermatozoa, using Nomarski differential interference microscopy at 1000 x. All measurements were made using a calibrated eyepiece micrometer.

RESULTS

Light microscope studies: sperm dimensions

Table 1 shows the sperm dimensions recorded for 100 *Macrotis lagotis* spermatozoa.

TABLE 1. — Dimensions of mature epididymal spermatozoa of *Macrotis lagotis*

Sperm dimension	(μm)
Sperm nuclear length	13.2 ± 0.2
Sperm nuclear width	1.6 ± 0.1
Nuclear length: width ratio	8.25
Midpiece length	16.2 ± 0.1
Principal piece length	130.2 ± 0.1
Total sperm length	149.4 ± 0.1

Ultrastructure of mature epididymal spermatozoa

The sperm head of *Macrotis lagotis* is cuneiform-shaped but slightly dorsoventrally flattened (Fig. 1b-c). A deep longitudinal groove runs caudally along the ventral nuclear surface from where the neck is inserted. The nuclear groove is narrow cranially but widens caudally to accommodate the midpiece (Fig. 1a). The majority of the dorsal aspect of the sperm head overlies the anterior 3/5 of the midpiece. Serial transverse sections through the sperm head indicate the presence of a dorsal nuclear ridge which extends from the tip of nucleus to its caudal extremity, but it is not prominent cranially (Fig. 1d-h).

The nucleoplasm is primarily condensed. However, some transverse sections indicate nuclear indentations along the periphery of the ventral flanges of the nucleus (Fig. 1f). A thin layer of parachromatin-like material extends caudally from the rostrum for approximately two fifths the length of the nucleus, and consists of material which is distinct from that of the acrosome, but is less electron dense than the nucleus (Fig. 2f-g). Poor preservation of membrane ultrastructure makes it difficult to ascertain whether the parachromatin-like material is within or outside the nuclear membrane.

There is an original primary and a distinct secondary implantation fossa (Fig. 2f-g). The original implantation fossa is located deep within the sperm head and lined by a thick layer of material which appears to form a basal plate. In the vicinity of the second implantation fossa and surrounding the neck region, granular material which has an ordered substructure is present (Fig. 1f). Some micrographs also indicate the presence of electron dense material which is confined to the caudo-ventral surface of the sperm head in association with the midpiece, (Fig. 1a, f), however, this material is particularly prominent in immature spermatozoa (Fig. 3b). It appears to have a distinct "honeycomb" lattice substructure.

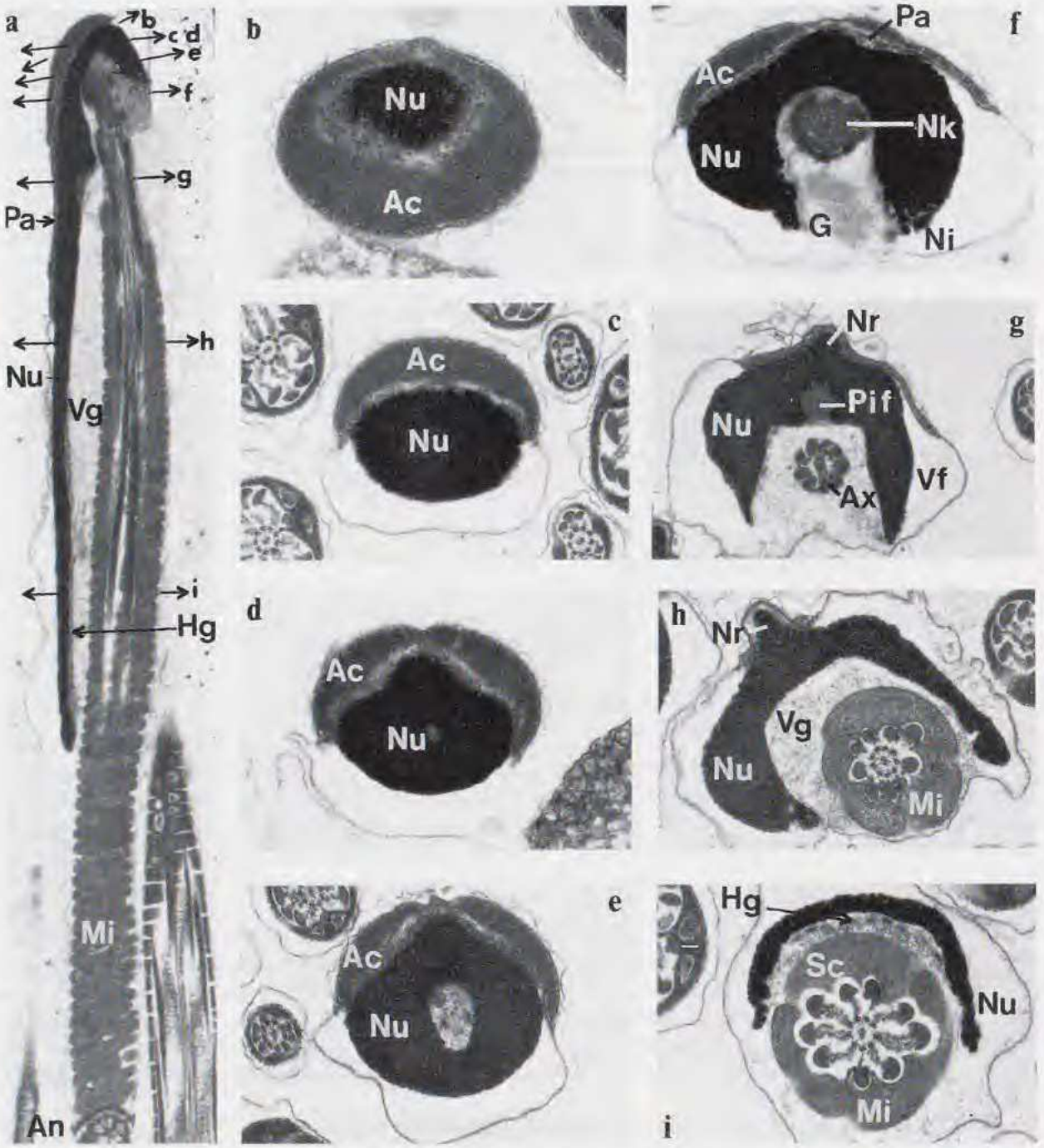


FIG. 1. — Electron micrographs of longitudinal sections (LS) and transverse sections (TS) of the nuclear and midpiece region of *Macrotis lagotis* cauda epididymal spermatozoa. **a:** LS of head and midpiece (x 11 200). **b-i:** TS through head to midpiece regions of *M. lagotis* spermatozoa as indicated in Fig. 1a. **a-e:** nuclear acrosomal region; b; x 21 000; c, x 21 000; d, x 21 000; e x 28 000; **f, g:** neck region; f, x 22 000; g, x 16 500. **h, i:** midpiece region; h, x 22 000; i, x 22 000.

The majority of the acrosomal matrix of the mature acrosome forms a rudimentary cap over the cranial dorsal nuclear surface (Figs 1a, 2f-g). However, it also extends dorso-caudally for two fifths the length of the nucleus in close association with the parachromatin-like material. Serial transverse sections through the caudal region of the acrosome indicate that the distribution of the acrosome conforms to the underlying profile of the nucleus (Fig. 1b-f). The cranial margin of the acrosome forms a lip at the cranial extremity of the nucleus (Figs 1a, 2f-g, 3a-b).

Longitudinal sections reveal that the axoneme terminates in a bifurcated structure, with the proximal centriole situated within the fork of the bifurcation (Fig. 2f). The dorsal arm of the bifurcation extends cranially past the proximal centriole and terminates surrounded by electron-lucent material. The ventral arm of the bifurcation is also surrounded by electron-lucent material but extends only adjacent to the proximal centriole.

The midpiece and underlying mitochondrial sheath are slightly flattened in transverse section (Fig. 2b). The ratio of the cross sectional diameters perpendicular and parallel to the centrally located axonemal fibres is 1.08 ± 0.03 (n=16). Mitochondria in transverse section are sculptured on their inner surfaces to accommodate the displaced outer fibres (Fig. 2b). Dense outer fibres 3 and 8 have an evenly circular contour, while the rest of the fibres has a semi-circular contour in cross section (Fig. 2b). The dense outer fibres of the cranial extremity of the axoneme, in the vicinity of the midpiece, are positioned close to the underlying axoneme doublet. However, in all transverse sections of the axoneme caudal to the nucleus, the dense outer fibres become widely separated. The separation is greatest between dense outer fibres 1, 5 and 6 and least between 3 and 8. Connecting lamellae in *M. lagotis* are double in nature except for fibres 3 and 8 (Fig. 2b). There is no diffuse granular material surrounding the outside of the mitochondrial sheath. However, transverse sections of the caudal midpiece region reveal an arc of doubled thicked membrane structures underlying the plasma membrane in the region of dense outer fibres 2, 3 and 4 and 7, 8 and 9 (Fig. 2b). In longitudinal sections these structures are located from the terminal caudal portion of the midpiece to a position approximately 10 mitochondrial whorls cranially. The annulus forms a fibrous ring below the most caudal mitochondria, and is joined to the fibrous sheath of the principal piece by an array of fine fibres (Fig. 2a).

Serial sections of the cranial region of the principal piece of *M. lagotis* spermatozoa are slightly flattened in cross section (Fig. 2c). The ratio of the cross sectional diameters perpendicular and parallel to centrally located axonemal fibres is 1.04 ± 0.02 (n=47). The fibrous sheath had only one fenestration (vacuole) in each rib on either side of the longitudinal columns (Fig. 2c). There is no typical endpiece, as the axoneme terminates before that of the fibrous sheath (Fig. 2d). Surrounding the fibrous sheath and underlying the plasmalemma there is also a layer of fine material; the accessory sheath (Fig. 2e).

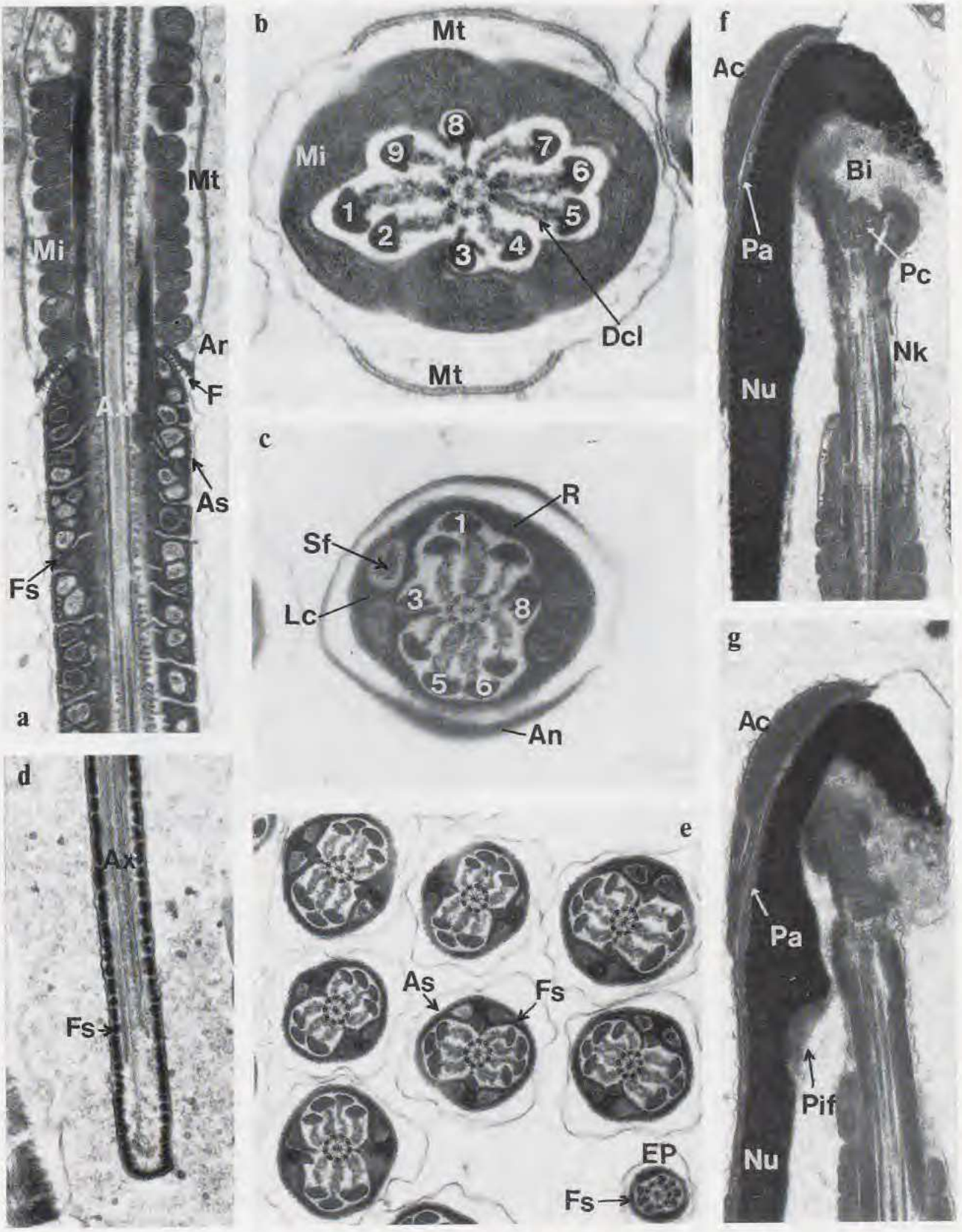
Epididymal sperm maturation

Light microscopic observations of spermatozoa in the lumen of the seminiferous tubule at spermiation and the caput epididymides indicate that the long axis of the nucleus ranges from perpendicular (Fig. 3a) to almost completely rotated parallel to the long axis of the axoneme (Fig. 3b). However, by the time spermatozoon reaches the cauda epididymidis the nucleus has fully rotated and is streamlined with the tail. The most notable change during epididymal transit is the dislocation and migration of the neck region from the original primary implantation fossa to a secondary implantation fossa located cranially (Fig. 3b). The dorso-cranial portion of the acrosome of caput epididymal spermatozoa appears button-like (Fig. 3c). However, during epididymal maturation this region appears to undergo compaction; becoming slightly flattened. The cytoplasmic droplet of the caput epididymal spermatozoon is eccentrically placed to one side

TABLE 2. — Ultrastructural characteristics of *M. lagotis* cauda epididymal spermatozoa and sperm maturation as compared with the Peramelidae and Dasyuridae. Bold type = *M. lagotis* unique character. Italics = *M. lagotis* shared character

Sperm Characteristics	Peramelidae	*Macrotis lagotis*	Dasyuridae
1. Sperm head shape	Complex	*Simple*	*Simple*
2. Presence of parachromatin	Extensive	?	?
3. Extent of ventral groove and relative position of neck insertion of mature spermatozoa	*Elongated and high*	*Elongated and high*	Moderate
4. Modification of nuclear periphery	*Only at lateral margins*	*Only at lateral margins*	Marked indentations on ventral surface
5. Lattice substructural material in region between the nucleus and the neck	No	**Yes**	No
6. Dorsal nuclear ridge	Yes but flattened	Yes but pointed	No
7. Head length (μm)	5.7 - 6.0	13.2 ± 0.2	9.5 - 12.7
8. Acrosome coverage as a fraction of the nucleus	Rostrum only	**2/5 of nuclear surface**	4/5 of nuclear surface
9. Shape of mitochondrial sheath in transverse section	Circular	**Slightly flattened**	Markedly flattened
10. Granular material surrounding mitochondrial sheath	Yes	*No*	*No*
11. Helical fibre network in midpiece region	*Absent*	*Absent*	Present
12. "Honey-comb" arranged lattice material on the caudo-ventral inner surface of the nucleus	Absent	**Present**	Absent
13. Arc of double thickened membrane underlining the plasma membrane of the caudal midpiece in the region of dense outer fibres 2,3,4 and 7,8,9	No	**Yes**	No
14. Midpiece length (μm)	10.7 - 19.3	16.2 ± 0.2	34.0 - 40.0
15. Neck region of axoneme bifurcated about the proximal centriole.	No	**Yes**	No
16. Shape of dense outer fibres in transverse section.	*Semi-circular*	*Semi-circular*	Circular
17. Connecting lamellae: (double or single)	*Double*	*Double*	Single
18. Shape of transverse section of principal piece	Diamond	**Slightly flattened**	Markedly flattened
19. Double or single fenestration in longitudinal columns	Double	*Single*	*Single*
20. Accessory sheath of principal piece	*Present*	*Present*	Absent
21. End Piece - lack of axoneme in terminal portion	*Yes*	*Yes*	?
22. Total sperm length (μm)	171.1 - 199.8	149.4 ± 0.1	218 - 255
Sperm maturation			
23. Neck dislocation from primary implantation fossa and cranial migration	*Yes, extreme*	*Yes, extreme*	No
24. Extent of head rotation	*Slight*	*Slight*	Marked
25. Acrosomal compaction	Not obvious	**Slight**	No
26. Increase in the electron density of the mitochondrial matrix material	Yes	*No*	*No*
27. Development of helical network surrounding the mitochondria and underlining the midpiece plasma membrane	*No*	*No*	Yes

FIG. 2. — Electron micrographs of *Macrotis lagotis* cauda epididymal spermatozoa. **a:** LS of midpiece and principal piece region illustrating microtubular structures at the caudal extremity of the midpiece and the annulus (x 22 500). **b:** TS through caudal extremity of midpiece (x 39 000). **c:** TS through annular region (x 36 000). **d:** LS of endpiece (x 28 000). **e:** TS through principal and endpiece regions (x 14 000). **f:** LS of the nuclear-flagellar connection, illustrating bifurcated neck-piece (x 24 000). **g:** LS of the nuclear-flagellar connection, illustrating primary implantation fossa (x 24 000).



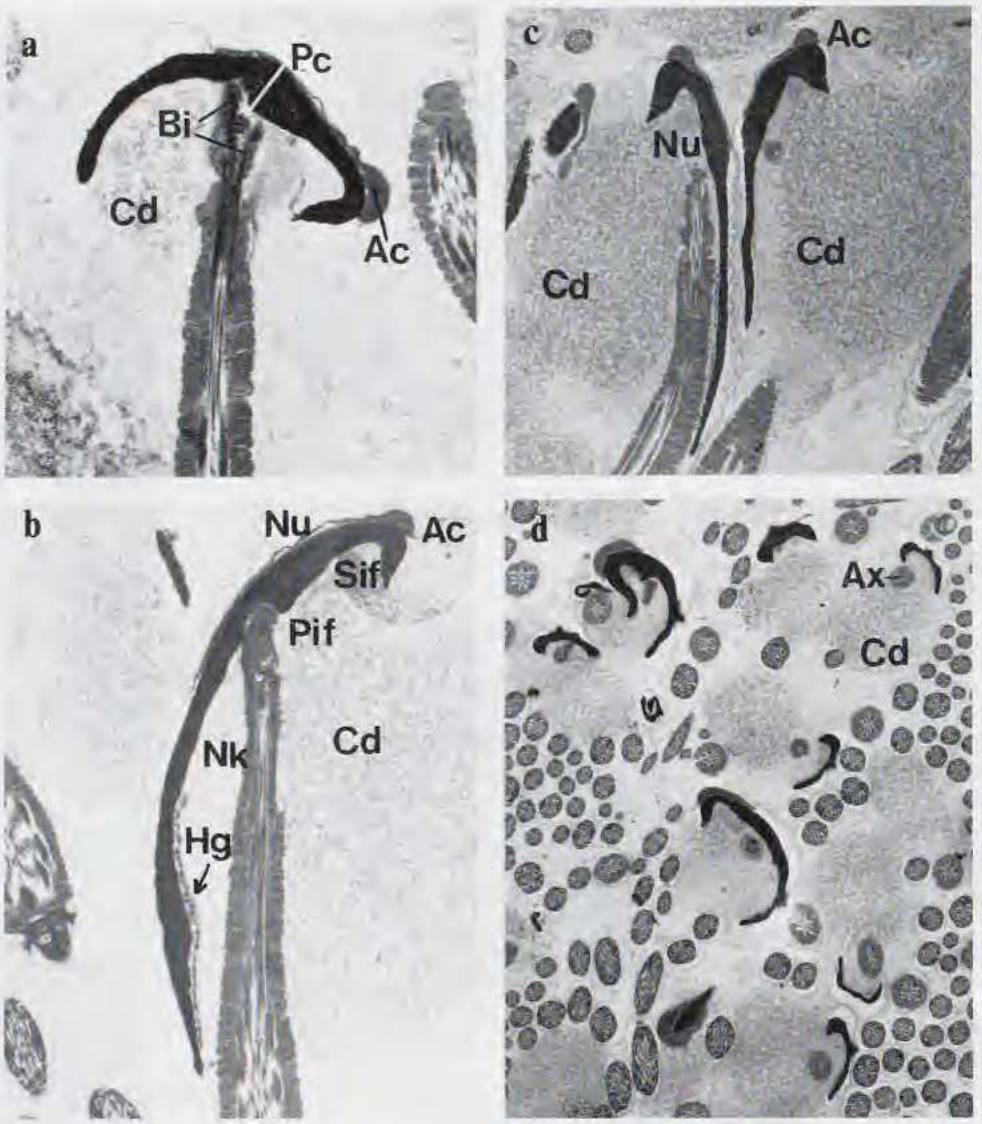


FIG. 3. — Electron micrographs of *Macrotis lagotis* immature caput epididymal spermatozoa. **a, b:** LS illustrating neck insertion into primary implantation fossa, button-like acrosome and cytoplasmic droplet. Also note bifurcated neck in a and honey-comb granular material in the caudal extremity of the nucleus in b. **c:** LS of nuclear-flagellar connection highlighting eccentric position of the cytoplasmic droplet. **d:** TS low power electron micrograph of immature spermatozoa. a, x 9 800; b, x 9 100; c, x 5 500; d, x 2 800.

Abbreviations for all figures: Ac, acrosome; An, annulus; As, accessory sheath; Ax, axoneme; Bi, bifurcated neck; Cd, cytoplasmic droplet; Dcl, double connecting lamellae; EP, endpiece; F, fibres of annulus attached to fibrous sheath; Fs, fibrous sheath; G, granular material about neck region; Hg, honey comb granular material; Lc, longitudinal columns; Mi, mitochondria; Mt, double thickened membrane; Ni, nuclear indentations; Nr, nuclear ridge; Nu, nucleus; Pa, parachromatin-like material; Pc, proximal centriole; Pif, primary implantation fossa; R, ribs of fibrous sheath; Sc, sculptured mitochondria; Sif, secondary implantation fossa; Sf, single fenestration of the fibrous sheath; Vf, ventral flange of nucleus; Vg, ventral groove ; 1-9, relative position of dense outer fibres.

of the midpiece and sperm head (Fig. 3a-d). The droplet appears to consist of two types of electron dense material. The first is easily recognised as composed of cytoplasmic membrane remnants, while the second is of less dense consistency (Fig. 3c).

DISCUSSION

Macrotis lagotis is the only extant member of the Thylacomyidae. Its spermatozoa are large compared to those of other mammals, but smaller than those of the Dasyuridae, Peramelidae and *Tarsipes rostratus*. Interestingly, while the total length of the *M. lagotis* spermatozoon is shorter than that of the other perameloids, its head length is up to twice as long.

Spermatozoa of *M. lagotis* display numerous unique ultrastructural characteristics which differentiate them both from the Peramelidae, (traditionally regarded as sharing a close phylogenetic relative), and the Dasyuridae. There are also numerous shared sperm characteristics between *M. lagotis* and the Dasyuridae and Peramelidae (See Table 2).

The head shape of *M. lagotis* is simple in profile, characteristically marsupialian [14] and without the complicated lateral concavities of typical Peramelidae or the hooked profile of the Vombatidae and Phascolarctidae. Like the Peramelidae, the ventral groove of sperm of *M. lagotis* is extensive, reaching almost to the tip of the nucleus. Similarly, both *Isoodon macrourus* and *M. lagotis*, have a nuclear ridge or keel on the dorsal surface of the sperm head, although the ridge of *I. macrourus* appears somewhat more flattened [14]. It is possible that such a structure could provide hydrodynamic stability during sperm motility.

The sperm nucleus of the Peramelidae is characterised by two chromatin components; an electron-dense and an electron-lucent component (or parachromatin). The nuclei of sperm of *M. lagotis* also appeared to contain parachromatin-like material; however, it is confined to the dorsal surface of the sperm head. It is still equivocal whether this material is part of the nucleoplasm. The occurrence of indentations along the periphery of the ventral flanges of the sperm nucleus appears similar to that described in the Peramelidae [14] but they are not as marked as those found in the Dasyuridae [15]. The anterior extremity of the ventral groove is also surrounded by material which is less electron dense than that of the parachromatin-like material. Unique to *M. lagotis*, so far as is known, this material had a lattice substructure.

During sperm maturation the neck of the axoneme dislocates from the primary implantation fossa to become located deep within the cranial extremity of the ventral groove. The secondary implantation fossa, however, does not possess a basal plate, and like other perameloid marsupials, connecting structures are not evident in association with the neck [14]. HARDING *et al.* [14] commented that lack of connecting structures might have meant that the head-flagella connection of the mature spermatozoon is not secure and that the stream-lined alignment of head and flagellum probably aided in preventing separation. Interestingly, this study also reveals electron dense material with a honey-comb lattice substructure on the interior surface of the caudo-dorsal sperm nucleus. This material is also apparent in nigrosin-eosin stained spermatozoa. Given that the sperm head of *M. lagotis* is found to be twice as long as that of other perameloid marsupials, it is possible that this material cements the caudo-dorsal extremity of the nucleus to the midpiece region, thus adding further strength to the head-flagella connection. One of the most striking features of the *M. lagotis* spermatozoon is the bifurcated morphology of the connecting piece, with the proximal centriole located within the fork of the bifurcation. This unusual structure appears to be unique to *M. lagotis* and a functional significance is elusive. Perhaps such an arrangement also strengthens the head-flagellum connection.

Acrosomal morphology of *M. lagotis* appears most similar to that of *T. rostratus* (Tarsipedidae). HARDING *et al.* [13] noted that the acrosome of *T. rostratus* covered the dorsal surface of approximately the anterior 2/3 of the nucleus. This observation compares with 2/5 of the nucleus of *M. lagotis* reported in this study. Secondly, the acrosome of both *T. rostratus* and *M. lagotis* forms a small lip at the cranial tip of the nucleus (HARDING, pers. comm.). The caudal portion of the acrosome of spermatozoa in the caput epididymis appears button like, but during epididymal transit the acrosome in this region flattens. Although acrosomal maturation of *M. lagotis* is only comparatively minor, perameloid and dasyuroid acrosomes appear fully compacted prior to epididymal transit.

HARDING *et al.* [14] had previously described the sperm flagellar ultrastructure of *M. lagotis*, noting the wide separation of dense outer fibres from the axoneme, presence of paired connecting lamellae, the single fenestration on either side of the longitudinal columns and the lack of a diamond shaped transverse flagellar section with a concave profile. However, owing to poor fixation and consequent quality of the material examined, midpiece ultrastructure was not described.

As for other perameloid marsupials [14], the dense outer fibres of *M. lagotis* in the cranial portion of the midpiece lie close to the underlying axoneme (Fig. 1h), but in caudal regions of the midpiece the dense outer fibres become radially displaced. However, the extent of the displacement in *M. lagotis* and the Peramelidae is not as marked as in the Dasyuridae and *T. rostratus* [13, 15]. As in the other perameloid marsupials [14] the shape of the dense outer fibres of *M. lagotis* sperm (except 3 and 8) is semi-circular in transverse section.

The shape of the caudal midpiece of *M. lagotis* in transverse section is slightly flattened, unlike that of the other perameloid marsupials which are essentially circular [14]. Also in common with the perameloids, dasyuroids and *Tarsipes rostratus*, the mitochondria are sculptured on their inner surface to accommodate the displaced dense outer fibres [13, 14, 15]. Spermatozoa of *M. lagotis* lack the granular layer surrounding the mitochondrial sheath, which is typical of the perameloid midpiece. However, there is an arc of double thickened membrane material underlying the plasma membrane in the region of dense outer fibres 2, 3 and 4 and 7, 8 and 9. These structures appear to be unique to *M. lagotis* . Observations from the midpiece region of *M. lagotis* sperm failed to detect the presence of an electron dense plate surrounding the midpiece or an underlying granular wedge; both apomorphies which characterize other perameloid spermatozoa [14].

The annulus of *M. lagotis* spermatozoa is "perameloid-like" in forming a fibrous ring caudal to the most posterior mitochondria and is joined to the fibrous sheath of the principal piece by an array of fine fibres [14]. Also similar to the sperm of other perameloids, dasyuroids and *T. rostratus*, the plasma membrane in the region of the annulus is not indented.

Compared to the other perameloids, dasyuroids and *T. rostratus* [13-15], transverse sections of the cranial principal piece are markedly less flattened: a feature unique to *M. lagotis*. However, unlike the other perameloids, but similar to the dasyuroids, the fibrous sheath of *M. lagotis* has only one fenestration in the rib of either of the longitudinal columns. In common with the Peramelidae, *M. lagotis* also has an accessory sheath surrounding the fibrous sheath of the principal piece, and the axoneme terminates before the fibrous sheath [14].

In conclusion, *Macrotis lagotis* spermatozoa display a number of unique characters that appear to separate them from the Peramelidae, Dasyuridae and *Tarsipes rostratus*. While a detailed cladistic analysis is currently in preparation, ultrastructural spermatozoal evidence on *M. lagotis* reported in this study, appears to support the present taxonomic status of the Family Thylacomyidae and confirm the close phylogenetic affinities (intermediate) with the Peramelidae, Dasyuridae and *T. rostratus*.

ACKNOWLEDGEMENTS

The authors thank Dr. David BLYDE of Western Plains Zoo for allowing access to Bilby testicular material and Mr. Tom GORRINGE, Department of Zoology, University of Queensland for printing the electron micrographs.

REFERENCES

1. BAVERSTOCK, P. R., FLANNERY, T., APLIN, K., BIRRELL, R. & KRIEG, M., 1990. — Albumin immunological relationships of the bandicoots (Perameloidea: Marsupialia) - a preliminary report. *In:* J. H. SEEBECK, P. R. BROWN, R. L. WALLIS & C. M. KEMPER, *Bandicoots and Bilbies*. Sydney, Surrey Beatty & Sons: 13-18.
2. CLELAND, K. W., 1955. — Structure of the bandicoot sperm-tail. *Australian Journal of Science*, **18**: 96-97.
3. CLELAND, K. W. & ROTHSCHILD, Lord, 1959. — The bandicoot spermatozoon: an electron microscopic study of the tail. *Proceedings of the Royal Society*, **150**: 24-42.

4. CLOSE, R. L., MURRAY, J. D. & BRISCOE, D. A., 1990. — Electrophoretic and chromosome surveys of the taxa of short-nosed bandicoots within the genus *Isoodon*. *In:* J. H. SEEBECK, P. R. BROWN, R. L. WALLIS & C. M. KEMPER, *Bandicoots and Bilbies*. Sydney, Surrey Beatty & Sons: 19-27.

5. CUMMINS, J. M., 1979. — Sperm maturation in the epididymis of the brindled short-nosed bandicoot, *Isoodon macrourus*. *Proceedings of the Annual Meeting of the Australian Society of Reproductive Biology* (Perth): 84.

6. CUMMINS, J. M., 1980. — Decondensation of sperm nuclei of Australian marsupials: effects of air drying and of calcium and magnesium. *Gamete Research*, **3**: 351-367.

7. DADDOW, L., 1986. — An abbreviated method of the double lead stain technique. *Journal of Submicroscopic Cytology* **18**: 221-4.

8. GROVES, C. P. & FLANNERY, T., 1990. — Revision of the families and genera of bandicoots. *In:* J. H. SEEBECK, P. R. BROWN, R. L. WALLIS & C. M. KEMPER, *Bandicoots and Bilbies*. Sydney, Surrey Beatty & Sons: 1-11.

9. HARDING, H. R., 1977. — *Reproduction in male marsupials: a critique, with additional observations on sperm development and structure*. Ph. D. Thesis. University of New South Wales, Sydney, Australia.

10. HARDING, H. R., CARRICK, F. N. & SHOREY, C. D., 1977. — Spermatozoa of Australian marsupials: ultrastructure and epididymal development. *In*: J. H. CALABY & C. H. TYNDALE-BISCOE, *Reproduction and Evolution; Fourth International Symposium on Comparative Biology of Reproduction*. Canberra, Australian Academy of Science: 151-152.

11. HARDING, H. R., CARRICK, F. N. & SHOREY, C. D., 1979. — Special features of sperm structure and function in marsupials. *In*: D. W. FAWCETT & J. M. BEDFORD. *The spermatozoon*. Baltimore-Munich, Urban & Schwarzenberg: 289-303.

12. HARDING, H. R., CARRICK, F. N. & SHOREY, C. D., 1983. — Acrosome development during spermiogenesis and epididymal sperm maturation in Australian marsupials. *In*: J. ANDRÉ, *The Sperm Cell*. The Hague, Martinus Nijhoff: 411-414.

13. HARDING, H. R., CARRICK, F. N. & SHOREY, C. D., 1984. — Sperm ultrastructure and development in the Honey possum, *Tarsipes rostratus*. *In*: A. P. SMITH & I. D. HUME, *Possums and Gliders*. Sydney, Australian Mammalogy Society: 451-461.

14. HARDING, H. R., SHOREY, C. D. & CLELAND, K. W., 1990. — Ultrastructure of spermatozoa and epididymal sperm maturation in some perameloids. *In:* J. H. SEEBECK, P. R. BROWN, R. L. WALLIS & C. M. KEMPER, *Bandicoots and Bilbies*. Sydney, Surrey Beatty & Sons: 235-250.

15. HARDING, H. R., WOOLLEY, P. A., SHOREY, C. D. & CARRICK, F. N., 1982. — Sperm ultrastructure, spermiogenesis and epididymal sperm maturation in dasyurid marsupials: phylogenetic implications. *In*: M. ARCHER, *Carnivorous Marsupials*. Sydney, Royal Zoological Society of New South Wales: 659-673.

16. HUGHES, R. L., 1965. — Comparative morphology of spermatozoa from five marsupial families. *Australian Journal of Zoology*, **13**: 533-543.

17. HUGHES, R. L., 1982. — Reproduction in the Tasmanian Devil. *Sarcophilus harrisii* (Dasyuridae,Marsupialia). *In:* M. ARCHER, *Carnivorous Marsupials*. Sydney, Royal Zoological Society of New South Wales: 49-63.

18. REYNOLDS, E. S. 1963. — The use of lead citrate at high pH and electron opaque stain in electron microscopy. *Journal ofCell Biology*, **17**: 20-28.

19. SAPSFORD, C. S. & RAE, C. A., 1969. — Ultrastructural studies on Sertoli cells and spermatids in the bandicoot and ram during movement of mature spermatids into the lumen of the seminiferous tubule. *Australian Journal of Zoology*, **17**: 415-445.

20. SAPSFORD, C. S., RAE, C. A. & CLELAND, K. W., 1967. — Ultrastructural studies on spermatids and Sertoli cells during early spermiogenesis in the bandicoot *Perameles nasuta* Geoffroy (Marsupialia). *Australian Journal of Zoology*, **15**: 881-909.

21. SAPSFORD, C. S., RAE, C. A. & CLELAND, K. W., 1969a. — Ultrastructural studies on maturing spermatids and on Sertoli cells in the bandicoot *Perameles nasuta* Geoffroy (Marsupialia) *Australian Journal of Zoology*, **17**: 195-292.

22. SAPSFORD, C. S., RAE, C. A. & CLELAND, K. W., 1969b. — The fate of residual bodies and degenerating germ cells and the lipid cycle in Sertoli cells in the bandicoot *Perameles nasuta* Geoffroy (Marsupialia). *Australian Journal of Zoology*, **17**: 729-753.

23. SAPSFORD, C. S., RAE, C. A. & CLELAND, K. W., 1970. — Ultrastructural studies on the development and form of the principal piece sheath of the bandicoot *Perameles nasuta* Geoffroy (Marsupialia). *Australian Journal of Zoology*, **18**: 21-48.

24. TEMPLE-SMITH, P. D., 1987. — Sperm structure and marsupial phylogeny. *In*: M. ARCHER, *Possums and Opossums: Studies in Evolution*. Sydney, Surrey Beatty and Sons & The Royal Zoological Society of New South Wales: 171-193.